

No kin discrimination in female mate choice of a parasitoid with complementary sex determination

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Discrimination against kin as mates, via genetic or environmentally derived cues of relatedness, can prevent inbreeding and thus enhance individual fitness and promote population survival. Sex in the parasitoid wasp *Cotesia glomerata* L. (Hymenoptera: Braconidae) is determined by one locus with multiple alleles, a mechanism termed single-locus complementary sex determination (sl-CSD). Under sl-CSD, haploid individuals are males, whereas diploid individuals are females when heterozygous at the sex determination locus but males when homozygous. In species with sl-CSD, inbreeding leads to increased incidence of matings between individuals sharing an allele at the sex locus and thus to increased diploid male production. Diploid males cause an undesirable sex ratio distortion and can be of inferior fitness. To evade these deleterious effects, species with sl-CSD are expected to avoid inbreeding. We investigated whether *C. glomerata* females discriminate against close kin as mating partners. We performed a mate choice experiment, which allowed us to distinguish between kin discrimination based on the perception of phenotype-related cues and kin discrimination based on the perception of cues associated with the developmental environment. As kin discrimination is often mediated through cuticular hydrocarbons (CHCs), we additionally examined composition of the CHC profiles of males. We found no evidence for discrimination against related or familiar males nor for differences in the CHC profiles of males. These results indicate that kin discrimination is not a relevant inbreeding avoidance strategy in *C. glomerata*. **Key words:** complementary sex determination, *Cotesia glomerata*, cuticular hydrocarbons, diploid males, genetic incompatibility, inbreeding avoidance, kin discrimination, mate choice. [*Behav Ecol* 21:1301–1307 (2010)]

Kin discrimination is the different treatment of conspecifics according to their genetic relatedness (Waldman 1987; Waldman et al. 1988). Discrimination between relatives and nonrelatives requires kin recognition, which can be based on the perception of phenotypic cues (direct kin recognition) or on cues associated with time, space, or environment (indirect kin recognition) (Pfennig and Sherman 1995). Kin discrimination is widespread throughout the animal kingdom and is involved in parental care, competition, cooperation, and mate choice (Waldman 1988). In mate choice, kin discrimination serves inbreeding avoidance (Foster 1992; Keller and Passera 1993) and can occur prior to mating (precopulatory kin discrimination) (Simmons 1989; Ode et al. 1995) or after mating (postcopulatory kin discrimination) (Sakaluk and Eggert 1996; Tregenza and Wedell 1998, 2002; Bretman et al. 2004). Precopulatory kin discrimination can be achieved through active avoidance of copulations with close kin, a behavior that occurs in the parasitoid wasp *Bracon hebetor* Say (Hymenoptera: Braconidae) (Ode et al. 1995). In this case, kin discrimination is based on environment-derived cues of familiarity, as *B. hebetor* females avoid mating with nonkin that developed on the same host, but do not discriminate against siblings emerged from a different host. Kin discrimination based on genetic relatedness occurs in the German cockroach *Blattella germanica* L. (Blattodea: Blattellidae) as well as in the two-spotted cricket *Gryllus bimaculatus* de Geer (Orthoptera: Gryllidae). Females of both species

prefer to mate with unrelated males rather than with siblings, apparently on the basis of direct cues (Simmons 1989, 1991; Lihoreau et al. 2007), which are most likely cuticle-derived non-volatile pheromones (Simmons 1990; Lihoreau and Rivault 2008). It is well known that the cuticle or rather the cuticular hydrocarbons (CHCs) are involved in insect chemical communication and recognition systems (Singer 1998; Howard and Blomquist 2005). To function as kin recognition cues, CHCs need to reliably reflect an individual's genotype (direct kin recognition) or the environment it experienced during development (indirect kin recognition) (Johansson and Jones 2007). Precopulatory kin discrimination is an adaptive inbreeding avoidance strategy as long as the costs incurred by a female mating with a close kin exceed those of discrimination or of forfeiting mating opportunities. Mating with close kin typically leads to inbreeding depression, that is, a decrease in the fitness of the offspring of close relatives due to increased levels of homozygosity, expression of recessive deleterious alleles, and a general decrease of genotypic variation (Charlesworth D and Charlesworth B 1987). Under systematic inbreeding, the deleterious effects on individual fitness increase the risk of population extinction (Saccheri et al. 1998; Frankham 2005; Wright et al. 2008).

In general, haplodiploid species are assumed to suffer less from inbreeding than diploid species. Most Hymenoptera have an arrhenotokous haplodiploid life cycle in which fertilized (diploid) eggs develop into females, whereas unfertilized (haploid) eggs develop into males, and deleterious alleles are purged in haploid males (Brückner 1978; Antolin 1999). Nevertheless, considerable inbreeding depression does occur in haplodiploid taxa (Antolin 1999; Henter 2003), especially in female-limited traits (Werren 1993). Furthermore, several genetic sex determination mechanisms that may add to the burden of inbreeding exist in the Hymenoptera, including

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single-locus complementary sex determination (sl-CSD) (Cook 1993; Heimpel and de Boer 2008). Under sl-CSD, sex is determined by one single locus with multiple alleles (Whiting 1940, 1943). Haploid (hemizygous) individuals are always males, whereas diploids develop into females when heterozygous at the sex determination locus and into males when homozygous (Cook and Crozier 1995). In species with sl-CSD, inbreeding increases the incidence of matings between individuals carrying an identical allele at the sex determination locus and thus the occurrence of diploid males (Cook and Crozier 1995). Diploid males can be costly in several ways. Diploid males often suffer from decreased viability or are effectively sterile (Cook 1993; de Boer et al. 2007), thus imposing a cost on their parents and on their mating partners. At the population level, the occurrence of diploid males skews the sex ratio toward males and can substantially reduce population growth and jeopardize population persistence (Stouthamer et al. 1992; Godfray 1994; Zayed and Packer 2005). To overcome the burden of diploid male production, species with sl-CSD are expected to have evolved mechanisms of inbreeding avoidance (Cook and Crozier 1995; Hein et al. 2009).

Cotesia glomerata L. (Hymenoptera: Braconidae) is a gregarious koinobiont endoparasitoid of the large white cabbage butterfly *Pieris brassicae* L., the small white butterfly *P. rapae* L. (Lepidoptera: Pieridae), and several other species in the subfamily Pierinae (Laing and Levin 1982). Zhou et al. (2006) showed that sl-CSD best fits as sex determination model in *C. glomerata*. However, diploid males can be functionally reproductive in *C. glomerata* (Elias et al. 2009), a rare phenomenon so far only described in the solitary hunting wasp *Euodynerus foraminatus* Saussure (Hymenoptera: Vespidae) (Cowan and Stahlhut 2004). In *C. glomerata*, inbreeding has no detectable influence on selected fitness-related traits, such as development time, body size, and brood size, but leads to a substantial distortion of progeny sex ratio (Zhou et al. 2007). The excess production of sons by inbred *C. glomerata* females is a form of inbreeding depression expected to fuel the evolution of inbreeding avoidance mechanisms that, however, have not yet been explored.

The question of whether and how kin discrimination contributes to inbreeding avoidance remains unanswered, despite an earlier anecdotal observation that not all *C. glomerata* females accepted a male at the natal patch but rather flew a short distance away or dropped from the leaf (Tagawa and Kitano 1981). Here, we ask whether females discriminate against kin as mates. To uncover whether discrimination relies on a direct or on an indirect basis of kin recognition, we separated the effects of genetic relatedness from those of the developmental environment. In parallel, we examined the CHC profiles of *C. glomerata* males to uncover potential differences between the surface recognition cues of unrelated and/or unfamiliar wasps. In line with previous studies on different insect species (Simmons 1989, 1990, 1991; Lihoreau et al. 2007; Lihoreau and Rivault 2008), we expected females to discriminate against kin in mate choice, thereby exploiting chemical contact cues that may signal relatedness or familiarity.

METHODS

Insects

Cotesia glomerata wasps were sampled by exposing *P. brassicae* larvae to parasitism in the field. Potted Brussels sprout plants infested with *P. brassicae* were distributed across 2 large cabbage-growing areas near Unter-Stammheim, Zurich, Switzerland (lat 47°38'N, long 8°46'E, 433 m above mean sea level), and exposed to the wasps for 2 days. Collections were performed 4 times each in the summers of 2007 and 2008. Recovered

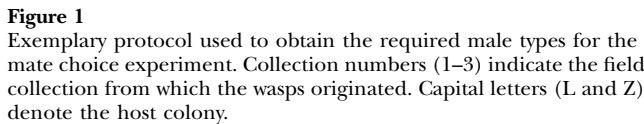
P. brassicae larvae were fed on cabbage leaves and kept in insect cages (30 × 30 × 30 cm) under a 16:8 h light:dark cycle at 21 ± 1 °C and relative humidity of 60%. Emerged wasps from the different collections were separately maintained under laboratory conditions. Wasps were cultured on *P. brassicae* larvae as hosts, and adult parasitoids were kept in insect cages (30 × 30 × 30 cm) in a climate chamber under a 16:8 h light:dark cycle at a temperature of 15 °C and 70% relative humidity. Access to honey and water was provided ad libitum.

Pieris brassicae larvae were reared on Brussels sprout plants (*Brassica oleracea* L. var. *gemmifera*) in an insectary under a 16:8 h light:dark cycle at 21 ± 1 °C and relative humidity of 60%. Adult butterflies were kept under the same climate regime and fed on a cane sugar solution (60%). Brussels sprout plants as oviposition substrate, artificial diet, and water were available continuously.

Mate choice

To investigate whether females discriminate against kin as mating partners and whether they use familiarity (development in the same host) or genetic relatedness as cues, we performed a dual choice arena experiment. In total, 4 possible types of male–female connection exist: familiar and related (FR), familiar and unrelated (FUR), unfamiliar and related (UFR), and unfamiliar and unrelated (UFUR), leading to 6 combinations of male–female connection to be tested. We had to simulate familiarity. *Cotesia glomerata* engages in superparasitism (Dorn and Beckage 2007), thus potentially allowing to obtain progeny from 2 females within the same host individual. However, the progeny of different females within the same host individual cannot be distinguished (Ruf D, personal observation). Alternatively, we used hosts genetically similar to each other as well as genetically diverse hosts to manipulate familiarity and to produce the male–female connections required for the mate choice experiment. The host can influence the CHC profiles of parasitoids (Howard 2001), and it is known that environment-derived chemical cues are exploited in familiarity-based recognition in insects (Gamboja 2004). We predicted that hosts from the same colony, due to their genetic similarity, have a similar influence on the CHC profile of the parasitoids and that these chemical cues originating from and/or influenced by the host are the most likely cues exploited in brood mate recognition in *C. glomerata*. Therefore, we treated wasps that developed in genetically similar hosts as familiar and that developed in genetically diverse hosts as unfamiliar. We established 2 distinct *P. brassicae* colonies (with ca. 50 adults per generation each) with little within-colony genetic variability. One colony (colony Z) had been maintained in the Applied Entomology laboratory (ETH Zurich, Switzerland) for over 10 years. The founders (ca. 60 individuals) of the other colony (colony L) originated from a long-running laboratory colony raised at the Plant Molecular Biology Department of the University of Lausanne (Switzerland).

In order to obtain the 4 types of connection and to exclude the occurrence of diploid males, an individually raised virgin female was allowed to parasitize *P. brassicae* larvae (L2) of both colonies (thus producing familiar and unfamiliar sons). After mating with an unrelated virgin male, the same female was allowed to parasitize larvae of one *P. brassicae* colony (thus producing daughters) following the protocol illustrated in Figure 1. Virginity of wasps was ensured by separating cocoons as soon as they exited the host and keeping the cocoons as well as emerging wasps individually in small plastic vials (1.5 × 3 cm). After oviposition, hosts were raised individually, and separated cocoons of *C. glomerata* were kept individually in plastic vials (1.5 × 3 cm). After emergence, wasps were provided with honey and water ad libitum.



Between January and December 2008, we ran 40 blocks, each block consisting of 6 mate choice trials covering the 6 combinations of male–female connection (240 choice trials in total). Within a block, the 6 tested females were daughters of the same once-mated female. The 6 males related to the females were brothers of which 3 developed in a host of the same colony as the females and 3 in a host of the alternative

As a reliable surrogate measure of body size (Elias J, Mazzi D, Dorn S, unpublished data), the length of the right hind tibia of all tested males was measured. Data on female mate choice and on the influence of male size on the choice were analyzed using likelihood ratio chi-square tests under the null hypothesis of no female choice. Data on mating latency were analyzed with a Wilcoxon-matched pairs test. Males that did not mate were assigned an arbitrary mating latency of 25 min (the maximum duration of a mating trial). Data on mating attempts and on male-female contacts were analyzed with a Mann-Whitney *U* test. Means \pm standard errors are given throughout.

The data from the FID analysis were subjected to principal component analysis. Data on the influence of host colony on CHC profiles were analyzed with discriminant analysis; data on the influence of relatedness on CHC profiles were analyzed with a *F*-test (families nested within blocks).

In total, 40 blocks with the 6 combinations of relatedness and familiarity within each block were tested. Four hundred and fifty-nine of the 480 (96%) males displayed courtship behavior, 381 (79%) had antennal contact with the female, and 314 (65%) attempted to mate. Matings occurred in 163 of the 240 choice tests (68%). Females did not discriminate between the

different male types in the 6 combinations (likelihood ratio chi-square test: all $G^2 < 2$, all $P > 0.2$; Figure 2). Mating latency did not significantly differ between the male types in the 6 combinations (Table 1). The number of mating attempts that successful males performed before female acceptance as well as the number of antennal contacts between successful males and females did not significantly differ between the different male types in the 6 combinations (Table 1).

Right hind tibia length of males ranged from 0.70 to 0.99 mm with a mean of 0.888 ± 0.003 mm. Male body size did not significantly influence female mating behavior: In 80 of 144 cases where mating occurred and the males differed in size, females mated with the larger male and in 64 cases with the smaller male (likelihood ratio chi-square test: $G^2 = 0.07$, $df = 1$, $P = 0.8$).

CHC profiles

The cuticle of *C. glomerata* males contained 19 hydrocarbons, both saturated and unsaturated, with chain lengths ranging from C_{21} to C_{35} (Figure 3). Principal component analysis reduced the data from FID analysis to one component, explaining 84% of the total variance. No significant differences were detected between either the CHC profiles of males developed in hosts of the 2 *P. brassicae* colonies (Discriminant analysis: Wilk's $\lambda = 0.993$, $P > 0.5$, 48.5% of all individuals were correctly classified) or the CHC profiles of males belonging to different families within the experimental blocks (F -test: $F_{17,34} = 1.34$, $P > 0.2$).

DISCUSSION

Cotesia glomerata females did not discriminate against siblings nor against familiar males as mates. Also, the CHC profiles of males did not noticeably vary depending on relatedness or familiarity. Furthermore, female mate choice was not influenced by male body size or male courtship intensity (duration of wing fanning, number of antennal contacts, and number of mating attempts). These results are intriguing because as a parasitoid with sl-CSD (Zhou et al. 2006), *C. glomerata* is expected to avoid inbreeding (Cook and Crozier 1995). The costs imposed by the production of diploid males might be smaller in *C. glomerata* than in other species with sl-CSD because diploid males can be fertile (Elias et al. 2009). Nevertheless, inbreeding leads to a substantial distortion of progeny sex ratio in *C. glomerata* (Zhou et al. 2007). Hence,

C. glomerata incurs severe inbreeding depression when matings between close kin are common (Zhou et al. 2007). Inbreeding is probably the most likely source of genetic incompatibility driving kin discrimination in mate choice (Tregenza and Wedell 2000). Hence, species with sl-CSD, where individuals sharing a sex allele should avoid mating with each other, provide informative model systems to investigate the role of genetic incompatibility in mate choice.

In the parasitoid wasp *C. glomerata*, we found no evidence of female discrimination against genetically related males or against males emerged from related hosts. Our approach for simulating familiarity by using 2 inbred host colonies may have dampened potential major differences. Regardless, the behavioral experiments do not suggest any form of discrimination in female mate choice, be it relying on CHCs or a different class of semiochemicals (e.g., male sex pheromones) (Keeling et al. 2004; Herzner et al. 2006) or a different sensory modality (e.g. visual cues). Our results thus indicate that *C. glomerata* females do not exploit either direct or indirect kin recognition in mate choice, consistent with recent evidence that neither females nor males of *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae) discriminate against kin in mate choice (Bourdais and Hance 2009).

Gregariousness of *C. glomerata* as well as of its main host in Europe, *P. brassicae* (Feltwell 1982; Laing and Levin 1982) and frequent occurrence of superparasitism (Gu et al. 2003; Dorn and Beckage 2007) lead to a high likelihood of mate encounters close to the natal patch. Using a related system with *C. glomerata* parasitizing the solitary host *P. rapae* in Japan, an early study suggested that a substantial proportion of parasitoid matings takes place at or close to the natal patch, with an estimated 60% rate of sibmatings (Tagawa and Kitano 1981), a value we consider biased upward for 2 reasons. First, superparasitism in the field is estimated at 25% in this system, with the actual rate likely exceeding this brood size-based estimate (Tagawa 2000). The proportion of field-exposed hosts superparasitized by different females was not recorded in the early study, which relied on cocoon clusters from field parasitized caterpillars (Tagawa and Kitano 1981). Hence, it neglected cryptic outbreeding occurring when offspring of different females develop in the same host. Second, it only considered matings occurring until departure from the site of emergence. Irrespective of the host system (*P. brassicae* or *P. rapae*), kin discrimination in mate choice would be predicted to be advantageous to *C. glomerata* females as they would benefit from the abundant mating opportunities close to the natal

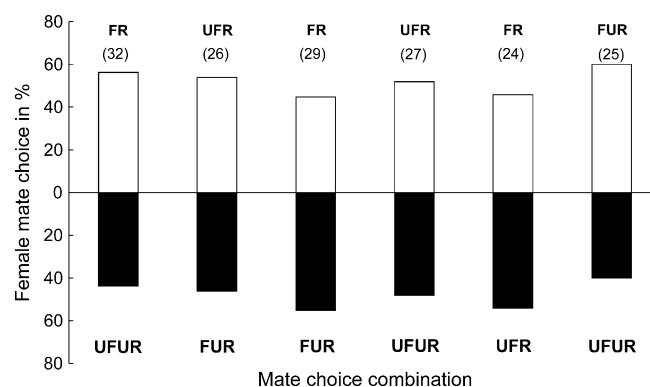


Figure 2

Mate choice of females in trials with males differing in relatedness and/or familiarity to the choosing female. Shown are matings with the different male types in percent of total matings per combination (given in parentheses). White bars represent matings with males of the type shown above the bars and black bars represent matings with males of the type shown below the bars. FR = familiar and related; UFR = unfamiliar and related; FUR = familiar and unrelated; UFUR = unfamiliar and unrelated.

Table 1
Results of the mate choice experiment

Mate choice combination	Mating attempts of successful males	Results of Mann–Whitney <i>U</i> -test	Female contacts of successful males	Results of Mann–Whitney <i>U</i> -test	Mating latency (s)	Results of Wilcoxon-signed ranks test
FR	2.4 ± 0.2	<i>U</i> = 111	1.8 ± 0.4	<i>U</i> = 102	872 ± 109	<i>Z</i> = −0.7
UFUR	2.8 ± 0.5	<i>P</i> = 0.56	1.7 ± 0.6	<i>P</i> = 0.38	1012 ± 108	<i>P</i> = 0.52
UFR	2.8 ± 0.6	<i>U</i> = 70	2.2 ± 0.6	<i>U</i> = 76	934 ± 122	<i>Z</i> = −0.2
FUR	2.7 ± 0.4	<i>P</i> = 0.49	2.4 ± 0.7	<i>P</i> = 0.71	939 ± 126	<i>P</i> = 0.88
FR	4.1 ± 0.6	<i>U</i> = 79	2.5 ± 0.5	<i>U</i> = 84	1042 ± 109	<i>Z</i> = −0.5
FUR	3.3 ± 0.6	<i>P</i> = 0.27	2.2 ± 0.5	<i>P</i> = 0.4	948 ± 113	<i>P</i> = 0.61
UFR	2.3 ± 0.3	<i>U</i> = 68	1.8 ± 0.4	<i>U</i> = 82	892 ± 126	<i>Z</i> = −0.01
UFUR	3.0 ± 0.6	<i>P</i> = 0.26	1.4 ± 0.3	<i>P</i> = 0.69	898 ± 125	<i>P</i> = 0.99
FR	2.4 ± 0.2	<i>U</i> = 69	1.4 ± 0.3	<i>U</i> = 57	940 ± 131	<i>Z</i> = −0.2
UFR	2.8 ± 0.5	<i>P</i> = 0.87	1.7 ± 0.4	<i>P</i> = 0.42	878 ± 130	<i>P</i> = 0.82
FUR	2.4 ± 0.2	<i>U</i> = 74	1.8 ± 0.2	<i>U</i> = 59	792 ± 124	<i>Z</i> = −0.3
UFUR	2.8 ± 0.6	<i>P</i> = 0.94	1.7 ± 0.6	<i>P</i> = 0.4	1000 ± 130	<i>P</i> = 0.76

FR = familiar and related, UFR = unfamiliar and related, FUR = familiar and unrelated, UFUR = unfamiliar and unrelated.

patch and simultaneously avoid inbreeding. However, kin discrimination does not appear to occur in *C. glomerata* mate choice.

It is not unusual that evidence for kin discrimination is lacking under circumstances where kin discrimination is expected. For instance, females of the parasitoid wasp *Nasonia vitripennis* Ashmead (Hymenoptera: Pteromalidae) do not adjust their offspring sex ratio depending on the relatedness of their mate nor depending on the relatedness of other females ovipositing in the same patch (Reece et al. 2004; Shuker, Reece, Taylor, et al. 2004; Shuker, Reece, Whitehorn, et al. 2004). On the other hand, genetic kin recognition does occur in several insect species (Simmons 1989, 1990, 1991; Lizé et al. 2006, 2007; Lihoreau et al. 2007; Lihoreau and Rivault 2008). One reason why kin discrimination is sometimes absent in spite of theory predicting its occurrence may be its evolutionary instability. Genetic kin recognition requires genetic polymorphism at one or more loci involved in the expression of the phenotypes on which recognition is based, but polymor-

phism will be eliminated by selection, unless sustained through high mutation rates or extrinsic processes (Gardner and West 2007; Rousset and Roze 2007). In addition to its evolutionary instability, kin recognition is costly as sensory systems consume considerable amounts of energy (Niven and Laughlin 2008). Many insects rely on sensory systems for host recognition and acceptance, nest mate recognition, gender recognition, and mate choice (Howard and Blomquist 2005), but such abilities can only evolve and persist as long as the derived benefits exceed the entailed costs. Accordingly, kin discrimination in female mate choice of *C. glomerata* can only have evolved provided that the benefits of inbreeding avoidance via kin recognition exceed its associated costs.

Behavioral strategies of inbreeding avoidance other than kin discrimination in mate choice, such as polyandry, might reduce the incidence of inbreeding (Tregenza and Wedell 2002). In several insect species, females are polyandrous (i.e., mate with more than one male), and unrelated mates achieve greater fertilization success than related males (Tregenza and Wedell 1998; Stockley 1999; Bretman et al. 2004; Bretman et al. 2009). In *C. glomerata*, females rarely remate in the laboratory (Ruf D, personal observation; Tagawa and Hidaka 1982; Tagawa et al. 1987), rendering polyandry an unlikely strategy of inbreeding avoidance in this parasitoid. One further strategy that might reduce inbreeding and ensure long-term survival of populations of *C. glomerata* is dispersal (Hein et al. 2009). In *C. glomerata*, 30% of the males and 50% of the females depart their natal patch prior to mating (Gu and Dorn 2003). With partial premating dispersal of both sexes, *C. glomerata* exhibits at least one behavioral strategy that can contribute to inbreeding avoidance by making kin encounters less likely. Additionally, by covering a large area during host search, females can scatter their eggs widely and limit the incidence of inbreeding (Wanner et al. 2007). Minor inbreeding depression and low chance of encountering relatives would substantially reduce selection pressure toward kin recognition in *C. glomerata*.

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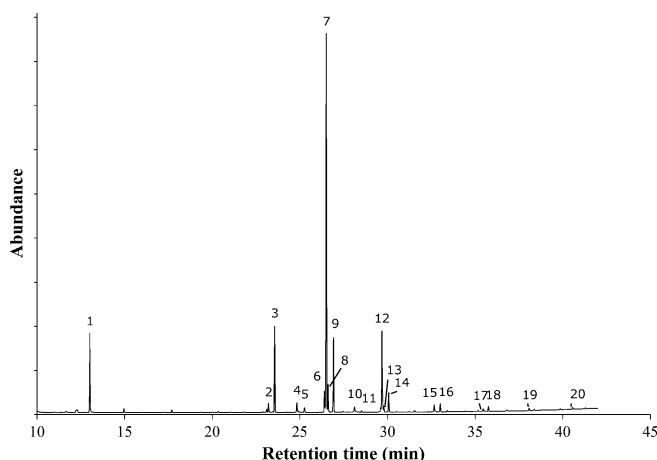


Figure 3
Representative gas chromatogram of CHCs extracted from an adult *C. glomerata* male with hexane. Peak identification: 1 = internal standard (C15), 2 = C21:1, 3 = C21, 4 = C22:1, 5 = C23:2, 6 = C23:1, 7 = C23:1, 8 = C23, 9 = C24:1, 10 = C24, 11 = C25:1, 12 = C25:1, 13 = C25, 14 = C27:1, 15 = C27, 16 = C29:1, 17 = C29, 18 = C31:1, and 19 = C35:1.

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